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THE EFFECTS OF β-PHENYLETHYLHYDRAZINE ON SEROTONIN, NOR ADRENALINE AND MONOAMINE OXIDASE IN CERTAIN TISSUES OF THE MALE RAT

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF PHARMACY

by

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ABSTRACT

An investigation was undertaken to determine the effects of β-phenylethylhydrazine (Nardil, Warner-Chilcott) on the serotonin, nor adrenaline and monoamine oxidase levels in the male albino rat. Brain, liver, lung and kidney were the tissues selected for study. Treated animals received 30 mg/kg. of the drug by intraperitoneal injection. Animals were sacrificed at 3, 20, 36 and 72 hours following the injection.

The drug markedly reduced the monoamine oxidase activity in all tissues investigated in 3 hours. Enzyme inhibition was most marked in the brain at this time. Liver monoamine oxidase returned to normal in 20 hours and reached an above-normal level in 72 hours. Kidney enzyme activity was normal in 36 hours. Brain showed a gradual increase in activity after 3 hours but had not reached the normal level by the end of the study. Lung continued to be markedly inhibited throughout the investigation.

The serotonin levels of brain increased as enzyme activity decreased. Kidney also showed elevated serotonin levels at all time intervals investigated. Liver serotonin levels were elevated at 3 and 72 hours. The serotonin concentration of lung did not change.

Nor adrenaline levels did not increase in the brain. A significant decrease in the catechol amine was observed in lung

in the 72 hour series. Kidney and liver nor adrenaline was elevated in the 3-hour and 36-hour series.

Theoretical aspects of these results are discussed.

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I. INTRODUCTION

With the advent in recent years of the group of drugs pharmacologically designated as tranquillizing agents, man has begun to probe into those areas of his being associated with anxieties, tensions and mental disturbances.

Studies of the pharmacological and biochemical effects of the tranquillizing and psychic energizing drugs have been undertaken in all types of mental disease ranging from mania to depression. The tremendous amount of knowledge which has been obtained is being expanded daily.

The effects readily seen with the psychotropic drugs appear to be mediated through the central nervous system and, therefore, much of the research has been concentrated on the biochemical effects in the brain.

This study was undertaken to evaluate the effects of such a drug on not only the brain of the rat but also on such tissues as the lung, liver and kidney. Measurements were made of the changes brought about by the drug on the monoamine oxidase activity, the concentration of serotonin and the concentration of nor adrenaline. The changes in activity or concentration were related to time after administration of the drug. In each of the tissues studied, certain definite changes were noted which were presumably related to the administration of the drug. An analysis of these changes is presented and suggestions are made as to the possible interpretation of these changes wherever possible.

II. LITERATURE SURVEY

1. Serotonin

A. Historical

The search for the elusive "vasoconstrictor substance" of blood by I. H. Page and his research group led to the crystallization of a highly active vasoconstrictor material from serum in 1947 (1-4). It was postulated by these workers that the structure of this substance was similar to that of tryptamine. The chemistry was finally elucidated by Rapport in 1949 and the material was found to be 5-hydroxytryptamine (5) complexed with creatine and sulfuric acid (5). Page named it serotonin because of its origin in serum and its tensing action on smooth muscle.

Erspamer, in Italy, had meanwhile been carrying out research on a smooth muscle stimulant extracted from the stomach mucosa of certain molluscs (6,7). He named the material 'enteramine' and believed it was a diphenolic or polyphenolic amine. In 1952 Erspamer and Asero found enteramine to be identical with 5-hydroxytryptamine (8). The structural formula of serotonin is shown in Figure 1.

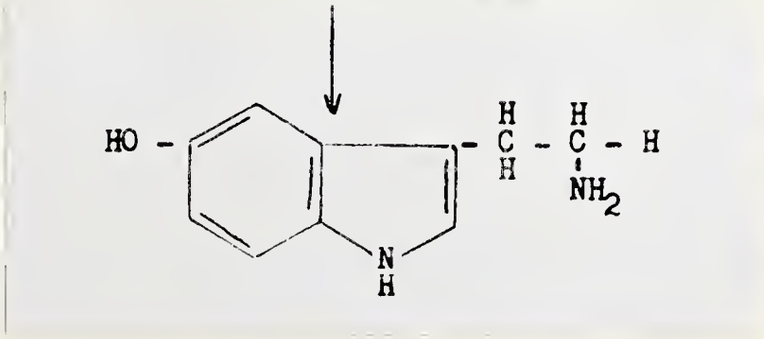

Figure 1
5-hydroxytryptamine (serotonin, 5-HT)

Discovery of serotonin in the brain (9,10) and the further finding that certain drugs associated with behavior can alter the concentration of this amine (11,12) has led to the postulate that serotonin may be considered a neuro-hormone.

Despite the mass of research papers published since the discovery of serotonin, the role which it plays in the body has yet to be conclusively determined. A more complete discussion of the evidence thus far elaborated is presented further in this report.

B. Occurrence

Serotonin has an extremely wide distribution in nature. It has been found in all classes of vertebrates (13,14) and in certain classes of molluscs (13,15) and arthropods (16-18). Serotonin is also found in the plant kingdom in classes of angiosperms (19,20) and in the banana plant (21).

In arthropods and angiosperms, the amine is confined to the venom of sting fluid (16-20). It would therefore appear that in these lower phyla serotonin has some survival value, particularly since applications of serotonin in very low doses (22) to exposed blister bases causes lasting pain.

Serotonin is found in many tissues of the mammal. Although tissue content varies from species to species, the smooth muscle of the gastrointestinal tract generally contains the greatest quantity of any tissue (13,23). The enterochromaffin cells of the gastrointestinal tract are believed to be the main site of 5-HT production in the mammal. High concentrations are also

found in the spleen of the rat and dog (9), and the lung (25, 27) and skin (25) of the rat. It is found in varying concentrations in most other tissues of the body (26). In the blood, 5-HT appears to be confined to the platelets (26-29).

The serotonin of the brain is not randomly distributed but is localized in definite areas (30). The primitive portions of the brain, such as the hypothalamus and mid-brain, contain the largest amounts, the cortex and cerebellum very little (30, 31).

Within the various tissues, serotonin is stored in a 'bound' form. Tissues, such as the gut, (which contains large amounts of serotonin) contract when small amounts of exogenous 5-HT are added to the medium (32). Since the large amount of serotonin normally present in the body is not physiologically active in toto, it would appear that serotonin must be stored in some type of cell or sub-cellular particle and released as necessary. In the gut and blood, the enterochromaffin cell and platelets satisfy this criteria. In other tissue sub-cellular particles, separate and distinct from mitochondria and containing 5-HT, have been described (33).

C. Metabolism

The over-all metabolic scheme for serotonin is shown in Figure 2.

Tryptophan is converted to serotonin in the intact mammal but it is not known in which tissue hydroxylation occurs (35, 36). The enterochromaffin cells of the gastrointestinal tract

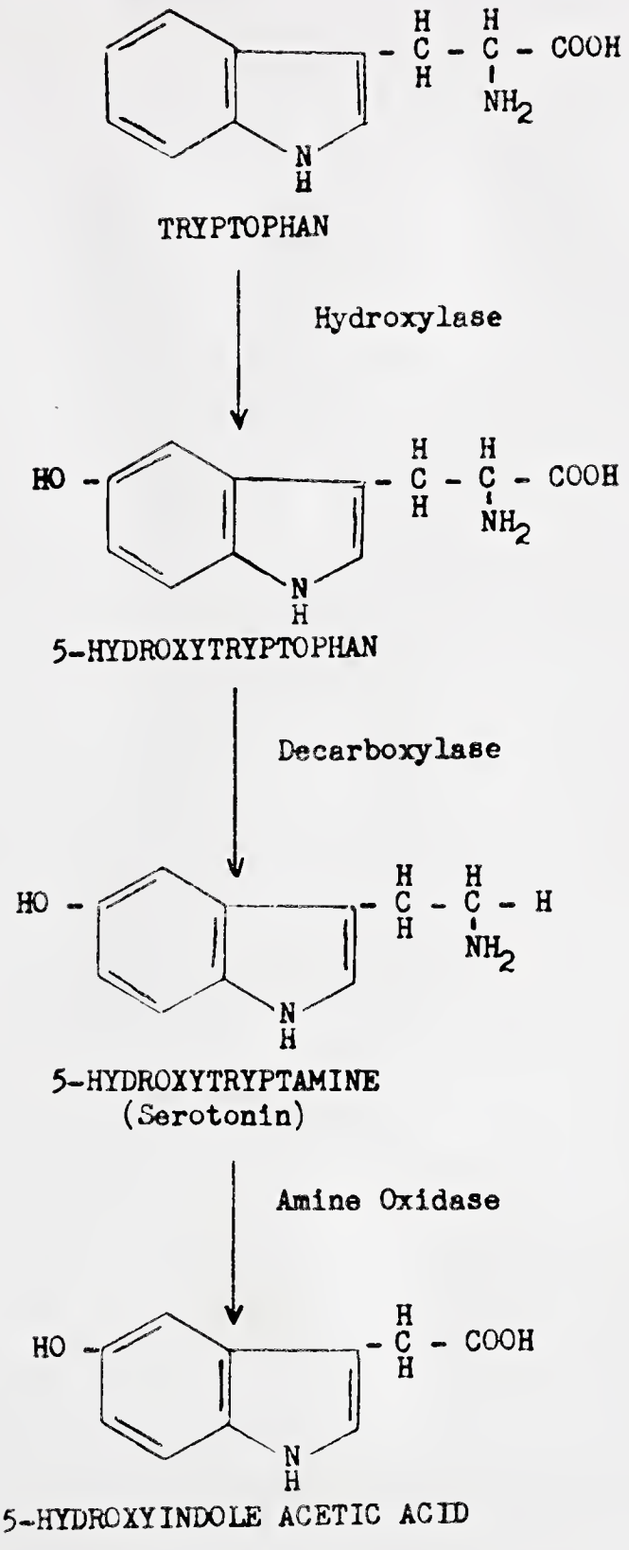

Figure 2

The Metabolism of Serotonin (34)

contain the necessary enzymes for serotonin biogenesis (27). Tumors of these cells result in the production of enormous amounts of 5-HT (37,38) whereas removal of large segments of the intestine, in various species, results in a decrease of serotonin metabolism (39-41). These facts would indicate that serotonin is produced in the gut. Blood and spleen 5-HT apparently originate from this same source (27).

Enzymes for the synthesis and destruction of serotonin are present in the brain and their distribution parallels the occurrence of 5-HT (30). Since serotonin cannot cross the blood brain barrier (35), although its immediate precursor 5-hydroxytryptophan can (42), it would appear that at least the final step in the synthesis of serotonin does take place in the brain.

The mast cells of the rat and mouse have also been shown to synthesize 5-HT (43).

D. Pharmacology

1. Cardiovascular actions. The effect of serotonin, in pharmacological doses, varies from species to species. In the dog, pressure usually falls with concurrent slowing of the heart rate; this is followed by a rise and finally a prolonged fall in pressure. Atropine or vagotomy prevent the initial drop, indicating that it is due to a von Bezold-like reflex. In the cat and rat, the same qualitative effects are seen as in the dog. However, vagotomy or atropine only partially abolish the initial fall in pressure. It would appear that

the fall in pressure is due to blockade of normal neurogenic vasoconstriction. The use of ganglionic blocking agents or destruction of the nervous system followed by 5-HT administration results in a prolonged pressor effect (44).

Injections of 5-HT into the pulmonary artery cause a rise in pressure not seen when injection is made into the pulmonary vein. Vasoconstriction and bronchioconstriction occur when serotonin is perfused through the lung of the cat or dog (44).

Intravenous injection of 5-HT produces effects on the heart which parallel the effects on blood pressure. Injections directly into the right atrium result in stimulation with a pronounced rise in blood pressure (44).

Serotonin stimulates the carotid sinus producing a powerful pressor effect (45).

The effect of 5-HT on the renal circulation appears to be mainly a constrictor one. Renal blood flow and glomerular filtration rate are reduced (46). Little et al. (47) found no change in glomerular filtration rate or renal blood flow. They did observe a significant decrease in renal excretion of sodium, potassium and chloride.

2. Anaphylaxis. The shock syndrome develops in guinea pigs within 60-150 seconds following administration of serotonin. The syndrome is similar to that produced by histamine except that death from 5-HT induced anaphylaxis is rare and tachyphylaxis rapidly develops (49). Another allergic manifestation

of 5-HT administration is the development of edema seen in the rat (50). Serotonin has recently been implicated in anaphylaxis in the mouse (54).

3. Isolated smooth muscle. Serotonin causes contraction of the smooth muscles of the gut, urogenital system, blood vessels, bronchial tree and the oestrous uterus (48, 51).

4. Gastric secretion. Smith (52) reports that 5-HT infusion does not stimulate gastric secretion. In fact, infusion was followed by an alkaline secretion rich in mucous. Administration of reserpine, to release endogenous 5-HT, produced results comparable to the exogenous infusion. Recently, Resnick and Gray (53) have found that physiological amounts of 0.01 N HCl cause release of 5-HT from the gut and postulate that 5-HT is, in some manner, necessary in the digestive process since the release of serotonin is pH dependent.

5. Antagonism. The most powerful antagonist of serotonin is lysergic acid diethylamide (32,55). Other antagonists, as found by Woolley and Shaw (32), are medmain, harmine, yohimbine, ergotamine and ergotoxine. Serotonin will also block its own actions at high dosage levels. Woolley and Shaw postulated what is known as the 'Serotonin Anti Metabolite Theory' based on the structural similarity of the antagonists tested to serotonin. This concept will be discussed later in this manuscript. Chlorpromazine has also been shown to be a serotonin antagonist (56).

E. Physiology

As yet, no definite physiological role can be attributed to 5-hydroxytryptamine, although vast amounts of research have been carried out in order to elaborate one. It is particularly difficult to attribute any physiological role to 5-HT in parts of the body other than the brain, despite the fact that the greatest amounts of 5-HT are found outside the central nervous system.

The theories for a physiological role, or roles, for serotonin in the periphery have recently been reviewed by Brodie (12) and Erspamer (27). Briefly, the theories state that serotonin is responsible for the following:

(1) The control of vascular tone and systemic blood pressure.

(2) The control of gastrointestinal tract motility.

(3) The regulation of water excretion by affecting kidney arterioles.

(4) The regulation of hemostasis by affecting blood vessels following release from platelets.

Brodie (12) states that since serotonin is present in the body in a bound, and therefore inactive form, it is doubtful if enough free serotonin is present to affect the vascular system as a whole, or renal function in particular. The pharmacological effects of 5-HT on the vascular system also tend to disprove this theory (44). Other workers (46,47) have shown that infused 5-HT lowers the glomerular filtration rate and

decreases renal blood flow. Decreases in the excretion of sodium, chloride, and potassium were also reported. However, doses used were pharmacological and results could not always be reproduced.

In an earlier paper from Brodie's laboratory (57), Haverback et al. showed that there was no change in bleeding and clotting time between normal animals and those whose platelets had been depleted of 5-HT by reserpine. Similar results were obtained using pyridoxine deficient chickens, which also have low 5-HT levels due to the inability to synthesize the amine (35).

The gastrointestinal tract contains the largest amount of serotonin but here again a physiological role cannot be readily ascertained. Peristalsis cannot be produced except with high doses of exogenous 5-HT (58). Serotonin does not stimulate gastric secretion (52) and the report that 5-HT is necessary for normal digestion (53) has yet to be reproduced.

Anaphylaxis, induced by serotonin aerosols, is rapid and transitory (49) and has been demonstrated only in the guinea pig. Serotonin depletion by reserpine has been shown to counteract anaphylaxis in the mouse (54), thus lending some support to its postulated role in the lung.

The presence of 5-HT in the central nervous system has, thus far, aroused the greatest interest of biologists. A number of independent groups have forwarded the suggestion that serotonin may play a role as a neurohormone (12,31,59).

Costa (60) defines a neurohormone as follows:

> By a neurohormone is meant either a transmitter substance or a substance that enhances or inhibits (modulates) the action of the actual transmitter. (61,62)

Costa presents the following evidence in favor of the view that 5-HT is a neurohormone, although as he points out, "The most decisive type of evidence, collection and identification of the amine after stimulation, has not been obtained."

Evidence that serotonin is a central neurohormone:

(1) The amine is localized in those areas of the brain that coordinate autonomic function with primitive patterns of behavior. The enzyme for the conversion of 5-hydroxytryptophan to serotonin is also present in these areas (63).

(2) The amine is present in a bound form.

(3) The enzyme for the metabolism of serotonin, monoamine oxidase, is present in all parts of the brain where 5-HT is found (30,63).

(4) The administration of serotonin into the brain elicits demonstrable effects. Serotonin does not readily penetrate the blood brain barrier (35); however, Shore et al. found that small, but significant, amounts of 5-HT entered the brains of mice following large doses. Reserpine-like effects, including sedation and potentiation of hypnotics, were observed. When administered in low doses, 5-hydroxytryptophan also produces sedation (63). With higher doses, excitement is observed (63,65). Blockade of monoamine oxidase also results in central increases of 5-HT and a corresponding hyperactivity

in the animal (12).

Thus, it can be seen that serotonin is present in the locale of its proposed function. The enzymes for its synthesis and destruction are also present. Interference with normal serotonin levels produces either sedation or excitement. On the basis of this evidence, Brodie and Shore (12,60) have postulated a theory for a physiological role for serotonin as a neurohormone. Serotonin is present in the central nervous system in a stored or bound form, Its physiological effects are produced by a release of a constant small amount of 'free' serotonin in the brain. Hess (67) has proposed that there are essentially two opposing systems controlling central nervous system function.

(1) The ergotrophic system which integrates those mechanisms that physiologically belong to body work. Activation of this system stimulates the sympathetic nervous system, increases skeletal muscle activity, induces arousal and activates psychic states.

(2) The trophotropic system integrates mechanisms that have a protective and assimilative function. Activation of this system stimulates the parasympathetic system and decreases motor activity and sensitivity to external stimuli and produces a drowsiness akin to natural sleep.

Brodie contends that 5-HT is the hormone concerned with control of the trophotropic system, and nor adrenaline the hormone controlling the ergotrophic system. Thus the low levels of free serotonin, normally present in brain, act as a homeostatic

mechanism. Release of serotonin by reserpine results in an increased 'free' serotonin concentration, although net serotonin stores are decreased. The trophotropic system is stimulated and tranquillization, followed by depression, occurs.

An increase in serotonin levels to amounts far greater than those found physiologically results in an effect opposite to that seen when administered after reserpine. Other hormones, for example, acetylcholine, are known to cause reversal of their usual actions when administered in excess. Serotonin also seems to exhibit this property.

Woolley and Shaw (32,68), while agreeing that 5-HT is a central neurohormone, have made other observations and have also postulated a physiological role for this amine. These workers have shown that, in vitro, serotonin causes the oligodendroglia cells of the brain to contract strongly. They reason that since the brain is a poorly vascularized organ, some mechanism is necessary to insure adequate circulation of extravascular fluids in order that oxygen, glucose and other nutrients may reach the brain cells and the corresponding waste products be removed. The 'glia' cells, by means of their pulsating action, could perform such a function.

Low levels of serotonin increase susceptibility to convulsions, and Woolley claims that this is due to anoxia produced by the non-functioning of the 'glia' cells. Hypoglycemia, as is seen with hyperinsulinism, produces hallucinations and

anxiety. These symptoms are also seen following administration of lysergic acid diethylamide (LSD), a potent serotonin antagonist. With this evidence, Woolley postulated that the effect of certain centrally acting drugs is due to the ability of these drugs to block 5-HT receptor sites. All of the drugs cited by Woolley do block serotonin when tested *in vitro* by pharmacological procedures.

In summary, it may be said that although serotonin satisfies sufficient criteria to be classed as a neurohormone, final definite evidence is still lacking as to its role in the body.

2. Nor Adrenaline

A. Historical

The importance of nor adrenaline as a naturally occurring amine is a relatively recent discovery. Despite the fact that Stolz, in 1904, synthesized nor adrenaline and made the first observations of its biological activity, little interest was shown in the compound until 1946 when von Euler demonstrated that it was the specific action substance of adrenergic nerves (69-71). Since this time, a remarkably large number of papers have been published on the metabolism, pharmacology and physiology of nor adrenaline.

The recent discovery by Marthe Vogt of the presence and unequal distribution of nor adrenaline in the brain has sparked even more interest in this compound (72). Further research by Vogt and others (12,72) has shown that certain psychomimetic drugs alter the levels of nor adrenaline in the central nervous

system thus stimulating further studies regarding the central role of nor adrenaline in the body.

The structural formula of nor adrenaline is presented in Figure 3.

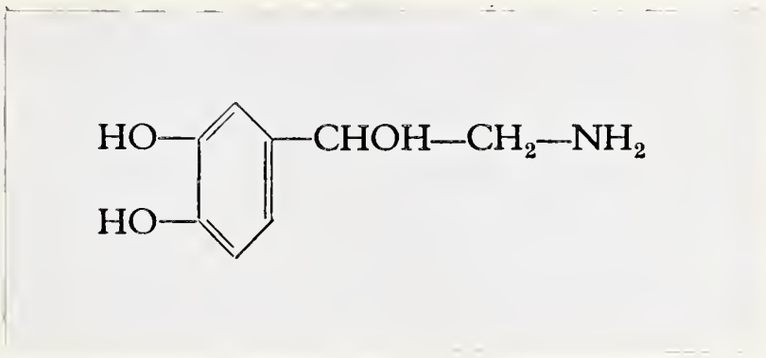

Figure 3

β (3,4 dihydroxyphenyl) β hydroxyethylamine
(nor adrenaline, nor epinephrine, levarterenol, NA)

B. Occurrence

Nor adrenaline is widely distributed in the animal kingdom, although its occurrence is apparently not as diverse as that of serotonin. It has been found in all vertebrates, molluscs and arthropods (69). Little research regarding its distribution in the plant kingdom has been carried out, but NA has been isolated from the banana plant (21).

In the mammal, NA is the chief pressor amine found in post-ganglionic adrenergic nerve fibres (69,71). Its presence in organs, other than brain, appears dependent on either adrenergic nerve supply or the presence of chromaffin tissue (69,71). Central NA follows a distribution pattern similar to

that of 5-HT (72). The adrenal medulla has varying amounts of nor adrenaline depending on the species (69). In general, in non-predatory animals, the amine content of the medulla consists mainly of adrenaline. Predatory animals, particularly those of the feline family, have more nor adrenaline. This is believed to be in direct relation to the predatory life these animals lead. Under these conditions, the quick release of a vasoconstrictor may be desirable to maintain blood pressure (69).

C. Metabolism

Synthesis. Although Blaschko and Holtz independently postulated the stepwise biogenesis of adrenaline in 1939, it remained pure speculation until the use of highly active C^{14} tracers provided more definite evidence (73). The steps in the formation of adrenaline and nor adrenaline, as they are now believed to occur, are presented in Figure 4.

Inactivation. Prior to 1957, it was generally believed that the principal means of detoxifying the catechol amines was deamination by the enzyme monoamine (MAO) oxidase (69). Other possible mechanisms have also been suggested in the literature (69). As early as 1940, it was observed that, in vitro, monoamine oxidase did not act rapidly enough to account for the breakdown of catechol amines (75). With the introduction of monoamine oxidase inhibitors, MAO was found to be relatively unimportant in the metabolism of catechol amines (76).

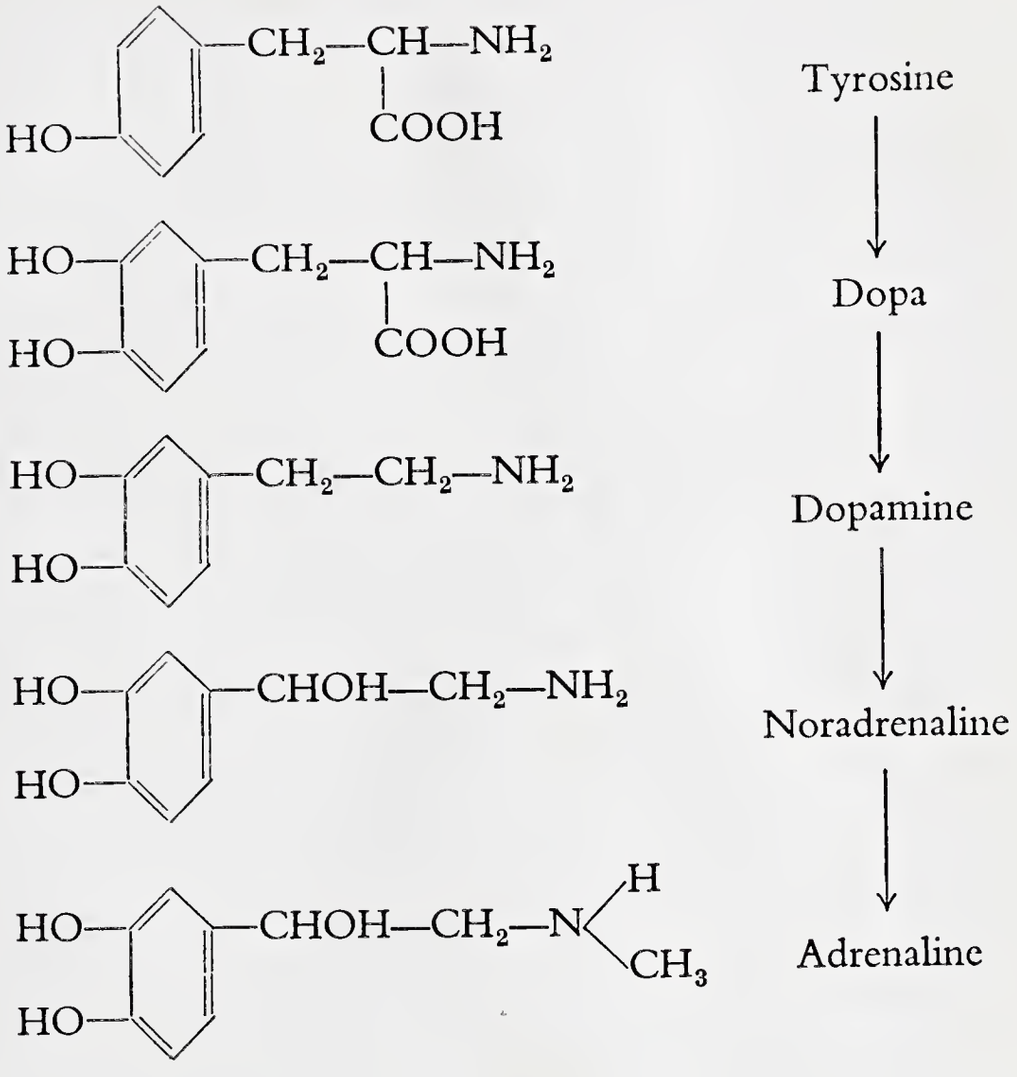

Figure 4

Formation of Catechol Amines (73,74)

The recent isolation and identification of the major metabolites of catechol metabolism as 3-methoxy-4-hydroxy-mandelic acid (77), metanephrine and normetanephrine (78), and 3-methoxy-4-hydroxyphenylglycol (79) led Axelrod (80) to believe that O-methylation is the most important pathway. The enzyme, catechol-O-methyl transferase (COMT), which O-methylates catecholamines and other catechols has been described (81). COMT has been found in all tissues examined including the central nervous system (81,82). Inhibition of COMT potentiates and prolongs the physiological effects of adrenaline and nor adrenaline, whereas inhibition of MAO does not (83).

Zeller (84) maintains that O-methylation is not applicable in every instance since brain and heart contain very little COMT. In these organs, inhibition of MAO results in protection and potentiation of catecholamines (85-87). This observation has been questioned (88).

Studies with radioactive adrenaline and nor adrenaline have led Axelrod to the following conclusion (shown in Figure 5) regarding the metabolism of these compounds (89-92).

Inhibition of MAO results in a greater excretion of non-deaminated catechols in the urine, whereas inhibition of COMT results in an increase of both free and deaminated products (92). It would appear that the route described is the principal method of breakdown for the catecholamines.

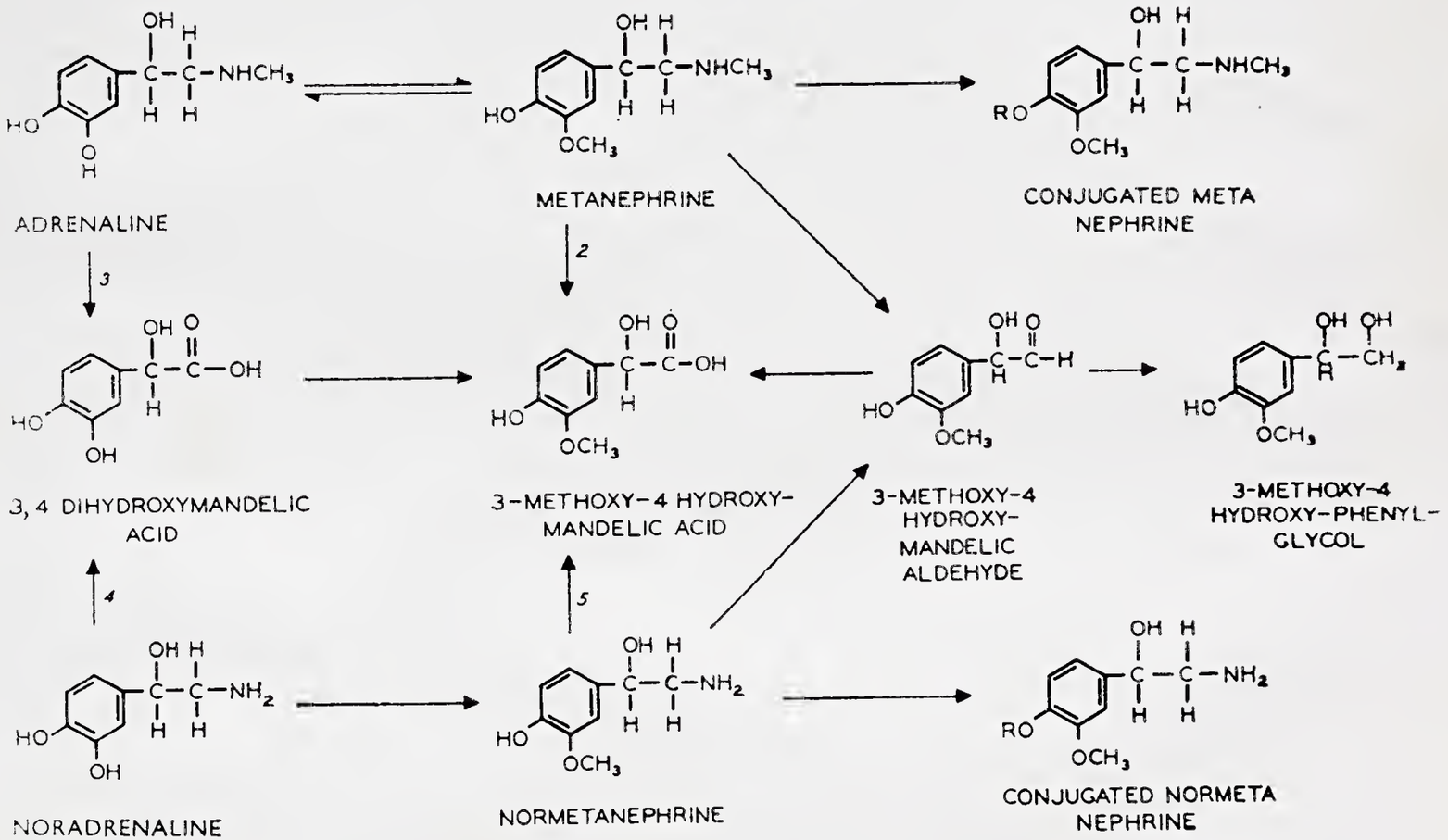

Figure 5

Metabolism of Adrenaline and Nor Adrenaline (92)

D. Pharmacology

1. Cardiovascular Effects

Nor adrenaline, when administered by slow intravenous infusion, causes the elevation of both systolic and diastolic pressure. Cardiac output remains the same, or decreases slightly, as peripheral resistance is augmented. Thus the main effect of NA is an increase in peripheral resistance due to vasoconstriction. The cardiac effects are masked by compensatory vagal reflex bradycardia. Adrenergic blocking agents can abolish the pressor effect without reversal of action as is seen with adrenaline (69-71).

2. Other Peripheral Effects

Other peripheral responses to nor adrenaline are not prominent and qualitatively mimic those of adrenalin, although higher doses usually must be used. There is an increase in oxygen consumption, hyperglycaemia and an increase in blood lactic acid. There is no eosinopenic response (69-71).

Intradermal injections cause sweating which is not blocked by atropine. There is an increased frequency of contraction of the pregnant human uterus, but effects upon other smooth muscles are not prominent (69-71).

3. Central Effects

The intravenous administration of large doses of nor adrenaline produces marked excitement in experimental animals (12). Large doses are necessary since catechol amines cross the blood-brain barrier with difficulty (12,70). The inhibition

of monoamine oxidase has been reported by some workers to cause an increased NA level in the brain (85-87,93). Serotonin levels also rise but increased activity has been correlated with the increase in NA (93). Other workers question this correlation (88). Administration of dihydroxyphenylalanine (DOPA), an immediate precursor of nor adrenaline, following inhibition of MAO, did not produce any psychological changes in humans (94), although it is claimed to arouse rats from reserpine stupor (134). Although the peripheral pharmacological manifestations of nor adrenaline are now well realized, the central effects of this amine are, at the present time, controversial and lacking in definite evidence.

E. Physiology

Nor adrenaline has been found to be the predominant sympathomimetic amine in mammalian sympathetic nerves and all organs innervated by them (71). The concept of nor adrenaline as the main adrenergic nerve transmitter is based on evidence which may be summarized as follows (69).

(1) NA has been demonstrated to occur specifically in adrenergic nerves.

(2) Degeneration and regeneration of adrenergic fibres is accompanied by the disappearance and reappearance, respectively, of NA.

(3) Stimulation of adrenergic nerve fibres results in the release of NA into the blood stream.

(4) The effects of such release show the characteristic action of NA directly on the stimulated organ.

(5) NA is excreted in the urine in both the normal and adrenalectomized animal.

(6) Drugs mimicking the action of NA have the same effect on organs as the released transmitter substance following adrenergic nerve stimulation.

(7) The biosynthesis of catecholamines in a number of sympathetic nerves proceeded only as far as nor adrenaline (73).

Nor adrenaline is present in a number of tissues, particularly the adrenal medulla, where it is stored in a granuole separate and distinct from that of adrenaline (95). Species variations in NA content of the medulla have already been mentioned, as well as the postulate as to why they occur.

It appears that whereas adrenaline functions as the great emergency hormone, the functions of NA are three-fold.

(1) As the adrenergic nerve transmitter.

(2) As a hormone.

(3) As the immediate precursor to adrenaline.

The physiology of NA in the central nervous system is infinitely more complex. Brodie contends that NA is neurohormone affecting the ergotrophic system of Hess (12). In support of this view, a great deal of evidence has been collected.

Costa et al. (60) has defined a neurohormone, and nor adrenaline appears to satisfy the criteria set out by Costa. Nor adrenaline is present in the brain in a bound form, and is localized in those areas of the brain coordinating autonomic function(72). The enzyme for the conversion of dopamine to nor adrenaline is also located in the brain (96). Both monoamine oxidase and catechol-0-methyl transferase, enzymes which play a role in the detoxification of nor adrenaline, are present in the central nervous system (30,63,81,82).

Catechol amines do not normally cross the blood brain barrier except in large doses (12,70). Marked excitement has been observed when such doses are administered intravenously (12,70). Increasing the NA content of the brain by the use of MAO inhibitors also produces excitation of central origin (85-87,93,97). Serotonin levels are also increased by the use of such drugs but Brodie (93) and Spector (97) have correlated pharmacological manifestations of the drugs with the rise in NA levels.

Costa et al. (60) have selectively released NA from the bound form using α-methyl-m-tyrosine (MMT). By previously administering a MAO inhibitor to raise both NA and 5-HT levels, the released NA should be in the free and active form only. Marked hyperactivity and excitement was noted. When the experiment was repeated, using reserpine which releases both amines, similar although less prominent results were obtained.

In dogs and cats, the use of MAO inhibitors did not raise

NA levels but selectively raised 5-HT levels. No increase in psychomotor activity was noted (97).

The evidence cited above, however, has been questioned. It is interesting to note that all evidence concerning the role of NA as a neurohormone has come from one group of workers. Other workers have performed similar experiments and have either obtained different, contradictory results or have interpreted the data in a different manner.

Vogt (98) has observed excitement in dogs following administration of iproniazid, an MAO inhibitor, despite an actual decrease in NA content of the central nervous system. Vogt (99) also reports that artificially raising NA levels does not alter behavior, and that administration of drugs prolonging sympathetic discharge resulting in signs of fear, rage and excitement produces a fall in catechol amine levels in the brain.

Funderburk et al. (88) have demonstrated that not all MAO inhibitors raise NA levels but all inhibitors tested did raise the central concentrations of 5-HT.

Inhibition of MAO followed by intraventricular administration of 5-HT, NA and adrenaline produced results implicating 5-HT as the prominent neurohormone. The effects of serotonin were both prolonged and potentiated by MAO inhibition, whereas those of both NA and adrenaline were not (100).

In 1960, Green and Sawyer developed a technique for the differential assay of bound and free amines in the brain (103). They also observed that administration of a MAO inhibitor did

not alter the concentration of free NA in the brain, although bound NA, and thus total NA, levels did increase (103). Green and Erickson have shown that the ability of tranylcypromine, a potent MAO inhibitor, to arouse rats from a reserpine-induced stupor was not accompanied by any reversal of reserpine-induced depletion of brain NA. Also, the excitation elicited by reserpine when injected into rats, pretreated with tranylcypromine, was not accompanied by any marked increase of total NA in the free form (104). Recently, Green and Sawyer (101) and Green and Erickson (102) have repeated the above experiments and measured 5-HT concentrations. They have found a correlation between 5-HT concentration and behavior in rats following administration of tranylcypromine and reserpine in one case, and iproniazid and reserpine in another experiment.

Free 5-HT levels were elevated by both MAO inhibitors, whereas free NA levels were not. The increase in free 5-HT levels was found to parallel the arousal following reserpine sedation, and the excitement seen when the MAO inhibitors were administered prior to reserpine.

Green *et al.* (136) have also shown that repeated oral doses of MAO inhibitors do not alter NA levels in relation to observed pharmacological effects.

Doubt has been expressed by Axelrod (106) that MAO is the main enzyme for metabolism of NA in the nervous system. Normetanephrine has been found in the brain (107). Pyrogallol, a COMT inhibitor, has been found to raise NA levels centrally (108). Other workers have not observed an increase in NA

following pyrogallol (109) and no one but Axelrod has found nor metanephrine in brain.

Reserpine releases both NA and 5-HT in the central nervous system, levels of both amines decreasing to approximately 10 per cent in two to four hours (12). However, it is now generally conceded that the action of reserpine is due to the release of 5-HT and not of NA. The evidence is reviewed by Costa et al. (60) and may be summarized as follows.

(1) If rats are stressed by exposure to cold following reserpine administration, no change in central 5-HT concentration is observed. The NA levels decline as usual but sedation does not occur.

(2) In low doses dimethylaminobenzoyl methylreserpate depletes brain NA stores in rabbits but does not affect serotonin. No sedation is seen. When larger doses, which deplete 5-HT, are used, a pronounced sedation is observed.

(3) Alphamethyl-m-tyrosine (MMT) selectively depletes NA stores without producing sedation.

It would therefore appear that although NA is found in the brain and outwardly appears to have a physiological role in controlling brain function, definite evidence of such a role, and indeed, exactly what the role is, is still lacking.

3. Monoamine Oxidase (MAO)

A. Historical

The first mention of the enzyme now known as monoamine oxidase, dates back to 1910 when Ewins (110) isolated

p-hydroxyphenylacetic acid from the perfusion fluid of rabbit liver to which tyramine had been added. Hare, in 1928, corroborated this finding and named the enzyme involved "tyraminase"(111). Work in the 1930's on the enzymatic oxidation of aliphatic amines (112) and adrenalin (113) resulted in the terms "amine oxidase" and "adrenaline oxidase", respectively. In 1937, it was found that the three enzymes were one and the same (114-116). To differentiate the enzyme from diamine oxidase, Zeller (117,118) proposed the name "monoamine oxidase". It is by this name that the enzyme is referred to today.

B. Occurrence

MAO has been found in various tissues of vertebrates, echinoderms and molluscs (115,119). The mammalian liver and kidney are the most convenient and richest sources of the enzyme (112,115,116,120). The enzyme is located in the particulate matter of a homogenate, approximately 50 per cent being associated with the mitochondria and 25 per cent with the microsome fraction (121). Species vary as to the amount of MAO present in the various tissues but, in general, practically all tissues contain some MAO (122).

C. Properties

The action of MAO may be described by the following equation (111,114,116,123).

$$RCH_2NR'_2 + O_2 + H_2O \rightarrow RCHO + NHR'_2 + H_2O_2$$

In the presence of cyanide (0.003-0.001M) in *in vitro* measurement, one atom of oxygen is consumed per molecule of substrate (111,113,124). MAO is fairly specific. In the series of aliphatic amines, the lowest members are usually not attacked (112,115,125). The optimum chain length is reported as being butylamine (122), although the optimal chain length is not the same for the hepatic MAO of different species (125).

MAO cannot catalyse the deamination of diamines (115); however, diamines of 14-18 carbon atoms do undergo oxidation (126). Presumably this is due to the diamine chain acting as two monamine units (126,127).

In general, MAO will not attack aromatic compounds in which the amino group is attached directly to the ring (115). The rate of oxidation of aromatic compounds increases as the side chain increases (128).

D. Measurement

MAO can be measured by:

(1) disappearance of substrate (129,130);

(2) oxygen consumption (122,124);

(3) production of ammonia (124);

(4) production of peroxide (124).

For routine laboratory work, the manometric measurement of oxygen consumption is the most practical and can be made relatively quantitative (124).

E. Biological Significance

Early workers in the study of the metabolism of catechol amines (CA) found that MAO oxidized adrenaline (113,131). Since that time, the controversy has raged as to whether MAO was the enzyme chiefly responsible for the metabolism of catechol amines. In 1940, it was pointed out by Richter (75) that, *in vitro*, MAO does not act rapidly enough to account for the breakdown of adrenaline. Recently, the metabolites of adrenaline and nor adrenaline have been identified (77-79), and a postulate proposed as to the route of metabolism of these amines (80).

It would appear from this evidence that MAO is responsible for the secondary deamination of 0-methylated catechol amines. Zeller (84) maintains this may not be the case in the brain and heart.

Dopamine (hydroxytyramine), the immediate precursor of NA, has been found to be mainly metabolized to the corresponding acid (132). MAO may therefore perform an important biological role in the inactivation of dopamine since dopamine is present in many tissues of the body (73), and when administered exogenously, produces effects similar to those of adrenaline (133, 134).

MAO is apparently the chief means of metabolizing the indole amine serotonin (34,135). Serotonin is deaminated to 5-hydroxyindoleacetic acid which is excreted in the urine (34, 135).

4. Monoamine Oxidase Inhibitors

A. Historical

The use of monoamine oxidase inhibitors in clinical medicine began with the observation in 1952 that patients being treated with the anti-tubercular drug iproniazid (IPZ) were showing signs of central stimulation (137). Iproniazid was therefore abandoned in favor of its close chemical relative isoniazid. Later in 1952, Zeller (138) showed that IPZ was a potent MAO inhibitor. By 1954 the alkylhydrazine portion of the molecule was recognized as being responsible for the MAO blocking ability of the drugs and several other alkylhydrazines had been investigated for their MAO blocking ability (138).

Discovery that IPZ potentiated and protected stores of catechol amines and serotonin, in the years 1953-1956, resulted in the return of IPZ to the clinical world, this time in a new role, as an agent for the alleviation of depression (137,138).

B. Pharmacology

The central pharmacology of the MAO inhibitors has previously been discussed under the physiology and pharmacology of serotonin and nor adrenaline.

Conclusions that MAO inhibitors act by means of potentiating these amines are based on experimental evidence which shows that all of these compounds block MAO and raise amine levels. The pharmacological effects are not seen immediately, but are dependent on the increases of brain serotonin and/or nor adrenaline. Most MAO inhibitors are metabolized rapidly and

the pharmacological manifestations of their administration appear after the drugs themselves have been excreted from the body (137). The data concerning which amine, 5-HT or NA is responsible for the central excitation produced has previously been presented in this manuscript.

In addition to central excitation, MAO inhibitors also exhibit marked anti-convulsant effects. Rats pretreated with MAO inhibitors show increased tolerance to electric shock. Iproniazid and other MAO inhibitors reduce the frequency of grand mal seizures and normalize the EEG pattern in epileptics. Amine levels must be elevated in order for these effects to be observed (137,139). Current evidence suggests that 5-HT elevation is responsible since administration of 5-hydroxytryptophane and IPZ showed anti-convulsant activity, whereas DOPA and IPZ did not (139). The free, and not the total, amine levels apparently are responsible for this action (139). It is of interest to note that reserpine, which depletes central amine levels, has a facilitory effect on experimentally induced convulsions (137,139).

Monoamine oxidase inhibitors have also been found to lower standing blood pressure in man (137). An observation that the orthostatic hypotension, seen in man following chronic use of MAO inhibitors, resembled ganglionic blockade (140) resulted in the investigation of these compounds as ganglionic blocking agents. All MAO blocking agents tested were found to be ganglionic blocking agents, isoniazid was not. Harmine, a reversible

MAO inhibitor, was the only agent whose ganglionic blocking ability could be reversed (141). The authors concluded that since the only mechanism common to the agents tested was their ability to block MAO, ganglionic blockade must be a result of the enzyme inhibition. The actual mechanism, however, is not clear (141).

Alleviation of the pain of angina pectoris is seen when MAO inhibitors are employed. Again, the actual mechanism is not clearly understood. Coronary artery dilation, ganglionic blockade and inhibition of catechol amine release have been implicated (137).

5. β-Phenylethylhydrazine (Phenelzine, Nardil*)

A. Chemistry

The MAO inhibitor used in this study was phenelzine. Chemically, the drug is β-phenylethylhydrazine dihydrogen sulphate (Figure 6).

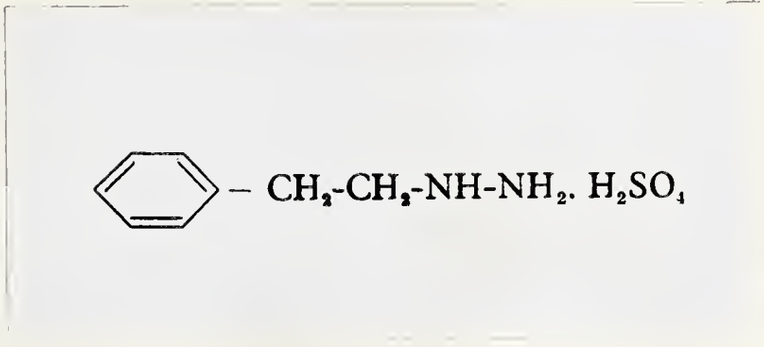

Figure 6

Structural formula of β-phenylethylhydrazine dihydrogen sulphate

*Warner-Chilcott.

B. Pharmacology and Therapeutic Uses

The pharmacology of phenelzine is basically the same as that of other MAO inhibitors. Nardil is used in the treatment of depression (142,143) and has shown clinical value in the management of angina pectoris (142). Side effects, when compared with other MAO inhibitors, are relatively low, and eventually subside without the necessity of discontinuing the drug (142).

The manufacturers recommend that Nardil be withheld in cases of liver disease, hypotension or impaired renal function (142).

The intravenous LD_{50} is 157 mg/kg, and the oral LD_{50} is 156 mg/kg (142).

Nardil is metabolized rapidly, less than 0.5 per cent of the total dose remains at the end of 20 hours (142).

III. EXPERIMENTAL

Throughout this investigation, male albino rats of the Sprague-Dawley strain, weighing between 150-200 grams, were used. The animals were maintained on Purina fox chow and tap water ad libitum, but food was removed twenty-four hours before sacrificing.

Treated animals received 30 mg/kg body weight of β-phenylethylhydrazine intraperitoneally in an aqueous solution. The volume of the injection did not exceed 0.25 ml. A series of animals was sacrificed by decapitation at 3, 20, 36, and 72 hours following the injection. The brain, liver, lungs and kidneys were removed and aliquots of these tissues were treated in order to measure either monoamine oxidase or serotonin and nor adrenaline levels.

1. Measurement of Monoamine Oxidase Activity

Enzyme activity was measured manometrically using a modification of a previously published procedure (144).

The tissues were rapidly removed and homogenized in 1.5 ml. of ice-cold Sorenson's phosphate buffer (0.067M), pH 7.2. A one milliliter aliquot was then removed and added to a Huston-Martin flask (152) containing 0.6 ml. of buffer and 0.2 ml. of 0.02M potassium cyanide. A filter paper disc, previously wetted with 0.2 ml. 10 per cent potassium hydroxide, was placed on the bottom of the flask. The flasks were then incubated at 37.5° for ten minutes. Tyramine, 0.2 ml. of 0.1M, was then added and the flasks were oxygenated for one minute.

Oxygen consumption was measured over the first 30 minutes using a Warburg apparatus. The enzyme activity was calculated as:

$$\frac{\mu l \text{ oxygen consumed/gm tissue/hour}}{10}$$

2. Extraction and Measurement of Serotonin

The tissues were rapidly removed and homogenized in 2 ml. N/10 hydrochloric acid. Approximately 300 mg. of lung and liver were taken from the animal with care in the dissection. One kidney and the brain were removed *in toto*. The entire kidney was used and the brain was bisected, with one-half only being taken for the extraction procedure. Care was taken to obtain the same half of the brain with a minimum of damage in all experiments.

Following homogenization, the acid homogenates were transferred to 40 ml. centrifuge tubes containing sufficient sodium chloride to saturate the acid solution. The homogenizer tubes were then rinsed with two 4 ml. aliquots of butanol* and the aliquots added to the centrifuge tubes. The tubes were shaken for 15 minutes and centrifuged for an equal period of time at 1000 r.p.m. A 4 ml. aliquot of the butanol layer was removed and added to a 40 ml. centrifuge tube containing 3 ml. N/10 HCl and 7 ml. heptane.* The centrifuge tubes were shaken for 5 minutes and centrifuged for 15 minutes at 1000 r.p.m. The organic layer was then removed and 1 ml. of the acid layer was taken for the measurement of serotonin. Standards in appropriate dilutions, and blanks were carried through the procedure.

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*Butanol and heptane solvents were treated as described under nor adrenaline extraction.

The 1 ml. of acid extract was placed in a quartz cell and 0.3 ml. 12N hydrochloric acid was added. The fluorescence of the resulting solution was measured immediately in an Aminco-Bowman spectrophotofluorometer (American Instrument Company). The sample was activated at 295 mμ and the resulting fluorescence read at 540 mμ. The blank reading was subtracted from the value recorded for samples and standards. A standard graph was prepared and the readings obtained on individual samples compared with this graph. The estimation of serotonin was reported as μg. serotonin/gm. fresh tissue.

3. Extraction and Measurement of Nor Adrenaline

Nor adrenaline was extracted by the same method as was used for serotonin. One ml. of the final acid extract was taken and treated according to the method of Shore and Olin (145).

A. Solvents and reagents

(1) n-butanol. Reagent grade n-butanol is purified by successive washings with 1N NaOH, 1N HCl and three washings with water. Following the washings with water, the pH of the butanol, as measured by indicator paper, should be above 3. The butanol is then shaken with 0.01N HCl. The aqueous phase is then removed, solid NaCl is added in excess, and the butanol is shaken until no more of the NaCl goes into solution. This serves to remove water dissolved in the butanol.

(2) n-heptane. Reagent grade heptane is washed successively with 1N NaOH, 1N HCl and twice with water.

(3) Iodine reagent, 0.1N Reagent grade iodine, 1.27 gm., is dissolved in 100 ml. of absolute ethanol.

(4) Sodium thiosulfate, 0.05N. Reagent grade $Na_2S_2O_3 \cdot 5H_2O$, 1.24 gm., is dissolved in 100 ml. of water.

(5) Acetate buffer, 2M, pH 5.0. To 2 volumes of 2M sodium acetate is added 1 volume of 2M acetic acid.

(6) Alkaline ascorbate solution. This is made immediately before use by adding 1 volume of an aqueous solution of ascorbic acid (10 mgm./ml.) to 2 volumes of 5N NaOH.

One ml. of pH 5 buffer was added to the 1 ml. sample followed by 0.1 ml. of iodine reagent. The oxidation was allowed to proceed for 6 minutes and was then halted by the addition of 0.2 ml. of thiosulphate solution. One ml. of alkaline ascorbate was then added and 45 minutes allowed to elapse. Nor adrenaline is converted to the highly fluorescent trihydroxyindole nor adrenolutine by this procedure.

Following development of the fluorescence, an aliquot was placed in a quartz cell, activated at 400 mμ and the fluorescence read at 520 mμ in the Aminco-Bowman spectrophotofluorometer.

Standards, and a blank, were carried through the procedure and results were calculated in a similar manner to that of serotonin.

4. Calculation of Results

Individual values were calculated as previously mentioned.

The mean of all individual values and the standard error of the mean were calculated (146) and are presented in tabular form.

Significance was tested by the "t" test and the probability of 0.05 was selected as the point of significance (147).

IV. RESULTS

The results obtained in five series of animals in which monoamine oxidase was determined, and a second five series of animals in which serotonin and nor adrenaline were estimated are presented in Tables I to IV.

In each series, a minimum of 12 animals was used to obtain the mean presented in the table. Individual results obtained in each experiment are included in the appendix.

The results are discussed according to the tissue involved.

1. Brain (Table I)

Table I presents a concise picture of the effect of phenelzine on the monoamine oxidase, serotonin and nor adrenaline concentrations in the rat brain.

Monoamine oxidase was significantly depressed by three hours after the intraperitoneal injection of the enzyme inhibitor. At this time, the enzyme had only 2.5 per cent of its normal activity. Monoamine oxidase levels continued to be depressed at all time intervals tested but had risen to approximately 78 per cent of normal in 20 hours and maintained this level at the subsequent times studied.

The serotonin concentration in the brain increased as the monoamine oxidase activity decreased. There appeared to be an inverse relationship between these components. Thus the greatest increase over normal in serotonin concentration noted was

at the 3-hour time period. In three hours the 5-HT concentration was approximately 62 per cent above normal. Serotonin concentrations remained significantly higher at all other time intervals investigated but gradually decreased so that in 72 hours 5-HT was 26 per cent above normal.

Nor adrenaline concentrations in the brain did not change significantly at any time period investigated.

A graphic representation of these results is presented in Figure 7.

2. Liver (Table II)

Liver presents a rather more complicated picture than does brain as can be seen from Table II.

Phenelzine significantly inhibited MAO in the liver. The concentration was reduced to about 68 per cent of normal in 3 hours. In 20 hours the enzyme concentration had returned to the normal level. However, in 72 hours the MAO concentration had risen significantly above normal.

The concentration of serotonin was significantly elevated in 3 hours and showed a further rise at 20 hours. In 36 hours the 5-HT concentration had returned to normal but a further significant increase was noted at the 72 hour time period.

Nor adrenaline concentrations also showed some variation. Concentrations of the amine were significantly elevated above normal at the 3 and 36 hour periods but were not statistically different from normal at the 20 and 72 hour periods. It can

be seen from the table (Table II) that figures for the 20 and 36 hour animals are practically the same. The 36 hour mean is higher than the 20 hour mean by just enough to make it significant ($P < 0.05$).

Figure 8 presents these results in graphic form.

3. Lung (Table III)

Monoamine oxidase activity was decreased to approximately 36 per cent of normal at 3 hours after injection of the inhibitor and the enzyme remained at this level throughout the study.

Serotonin levels did not change significantly.

The concentration of nor adrenaline was not significantly altered until 72 hours after the drug was administered. At this time the NA concentration was decreased to about 45 per cent of normal. This decrease was highly significant.

The results are presented in Table III and Figure 9.

4. Kidney (Table IV)

In this tissue, MAO activity had decreased at the end of 3 hours and remained significantly lowered after 20 hours. Recovery to normal occurred in 36 hours.

Serotonin levels were markedly elevated in 3 hours and remained elevated throughout the study.

Nor adrenaline concentrations exhibited a biphasic elevation at the 3 and 36 hour time periods. Normal values were observed at the 20 and 72 hour periods.

Table IV presents these results and they are shown graphically in Figure 10.

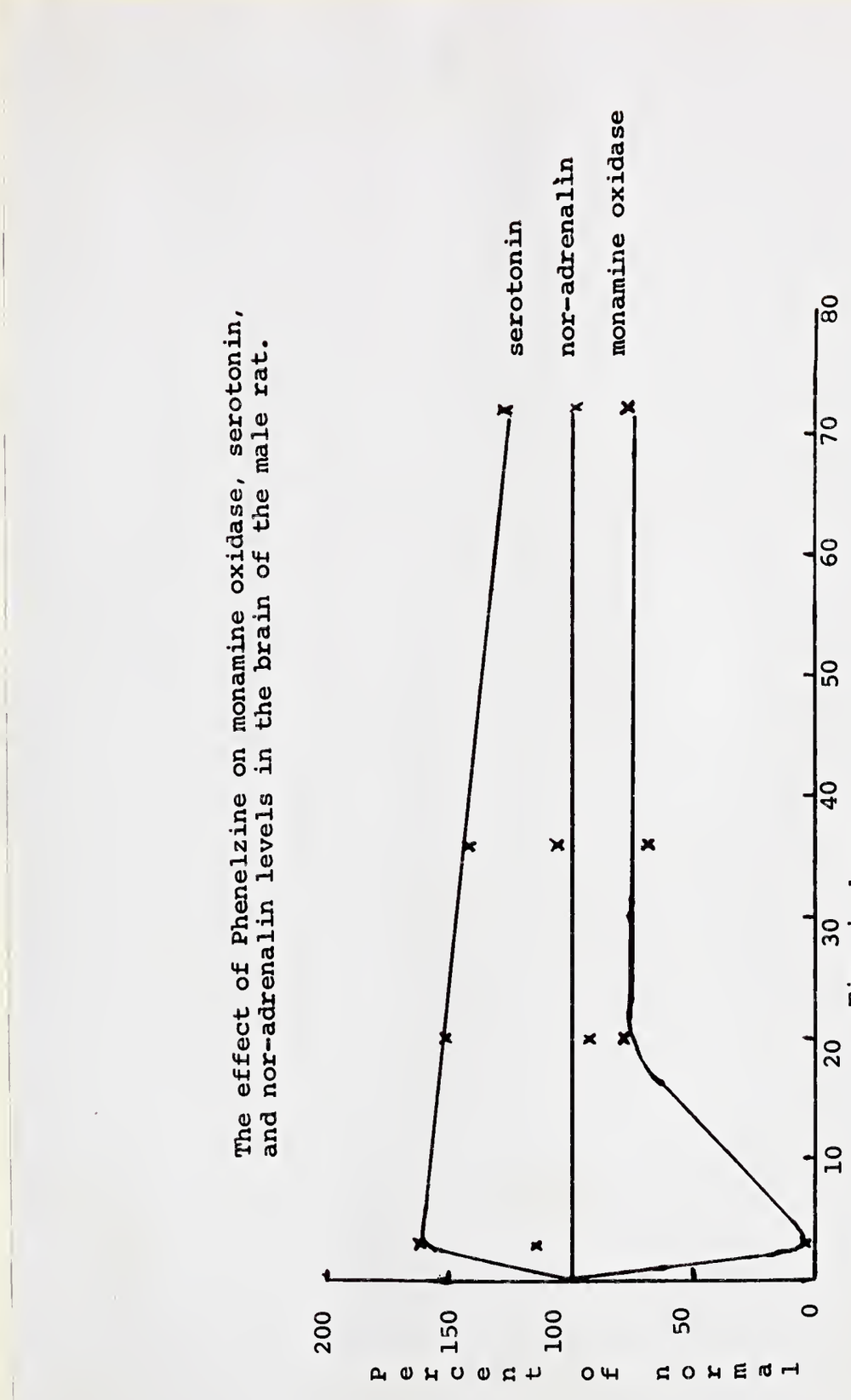
The effect of Phenelzine on monamine oxidase, serotonin, and nor-adrenalin levels in the brain of the male rat.
Percent of normal
200
150
100
50
0
10
20
30
40
50
60
70
80
Time in hours
serotonin
nor-adrenalin
monamine oxidase

Figure 7

TABLE I

THE EFFECT OF PHENELZINE ON MONOAMINE OXIDASE, SEROTONIN AND NOR ADRENALINE LEVELS IN THE BRAIN OF THE MALE RAT

Series	Monoamine oxidase	P	Serotonin	P	Nor adrenaline	P
Normal	43.78 ± 3.68		0.68 ± .03		0.32 ± .03	
3 Hour	1.10 ± 0.75	< .001	1.10 ± .06	< .001	0.37 ± .02	
20 Hour	34.28 ± 2.60	< .05	1.02 ± .07	< .001	0.29 ± .01	
36 Hour	29.44 ± 1.72	< .001	0.97 ± .04	< .001	0.34 ± .01	
72 Hour	33.19 ± 2.31	< .01	0.86 ± .02	< .001	0.31 ± .03	

(Significance was determined between normal and treated animals.)

Results are expressed as:

monoamine oxidase activity in $\frac{\mu l \text{ oxygen consumed/gm tissue/hour}}{10}$

serotonin and nor adrenaline in μg/gm.

The effect of Phenelzine on monamine oxidase, serotonin, and nor-adrenalin levels in the liver of the male rat.

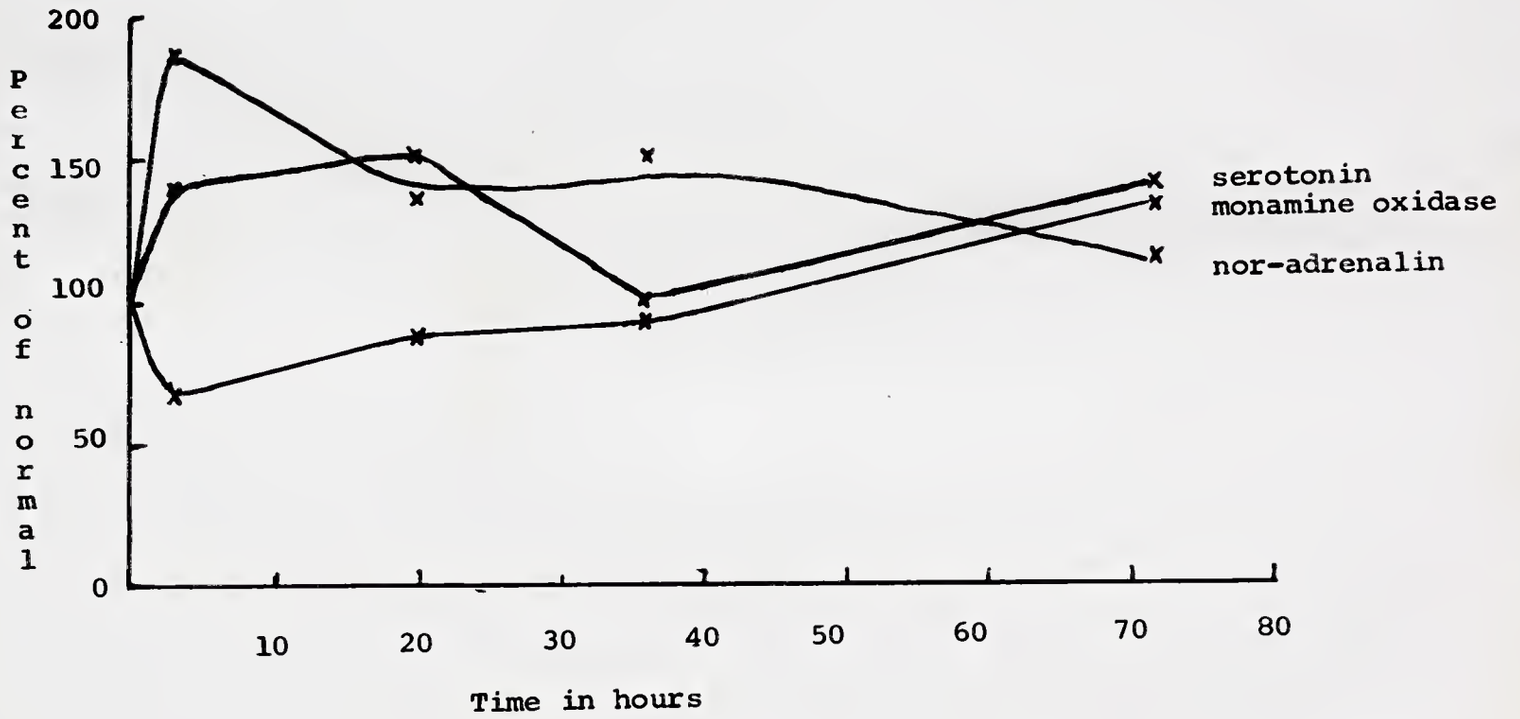

Figure 8

TABLE II

THE EFFECT OF PHENELZINE ON MONOAMINE OXIDASE, SEROTONIN AND NOR ADRENALINE LEVELS IN THE LIVER OF THE MALE RAT

Series	Monoamine oxidase	P	Serotonin	P	Nor adrenaline	P
Normal	68.73 ± 3.43		0.61 ± .03		0.14 ± .02	
3 Hour	46.39 ± 1.97	< .001	0.85 ± .10	< .02	0.26 ± .02	< .001
20 Hour	60.67 ± 4.56		0.92 ± .06	< .001	0.19 ± .02	
36 Hour	63.20 ± 5.59		0.60 ± .04		0.21 ± .02	< .05
72 Hour	91.43 ± 3.01	< .001*	0.86 ± .10	< .02	0.16 ± .03	

*Significantly higher.

(Significance was determined between normal and treated animals.)

Results are expressed as:

monoamine oxidase activity in $\frac{\mu l \text{ oxygen consumed/gm tissue/hour}}{10}$

serotonin and nor adrenaline in µg/gm.

The effect of Phenelzine on monamine oxidase, serotonin, and nor-adrenalin levels in the lung of the male rat.

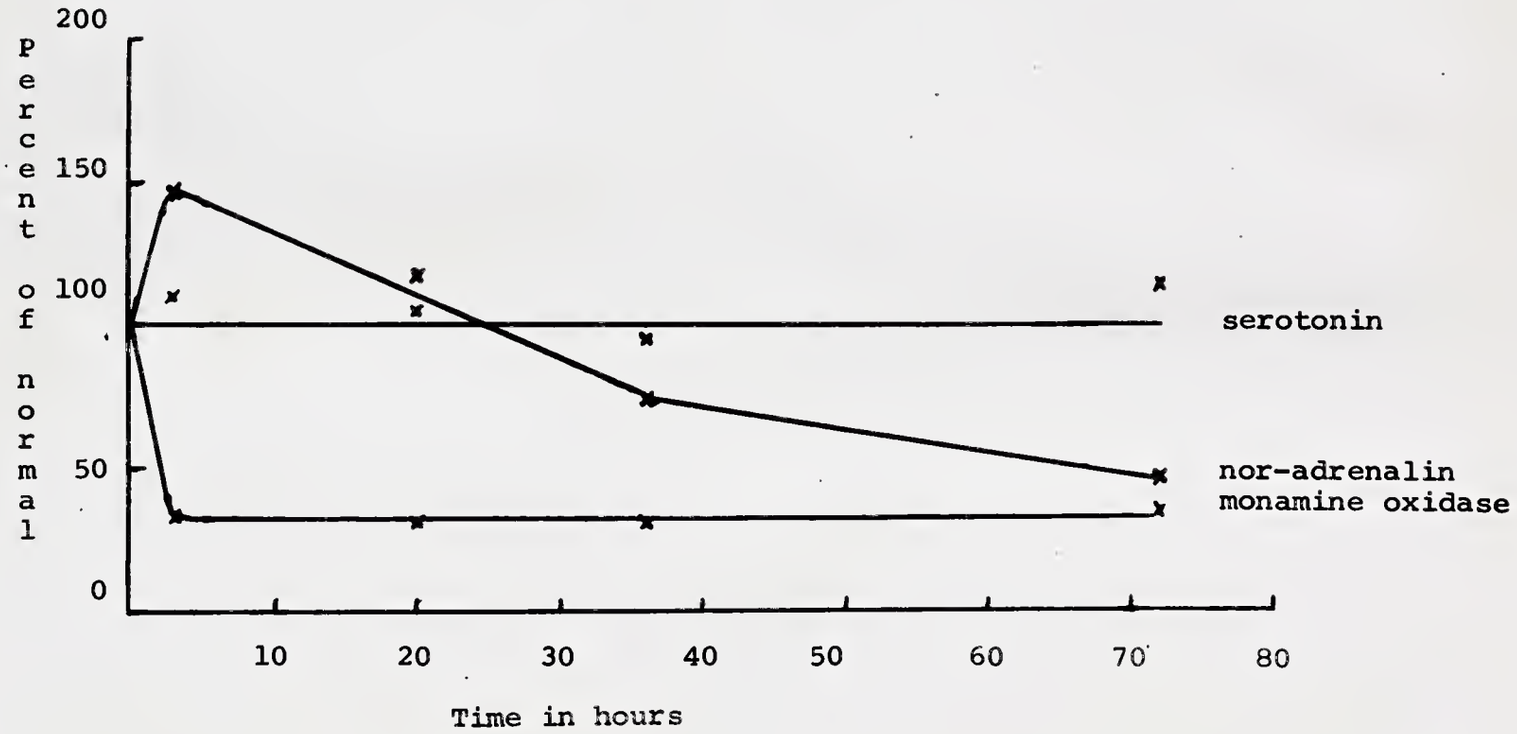

Figure 9

TABLE III

THE EFFECT OF PHENELZINE ON MONOAMINE OXIDASE, SEROTONIN AND NOR ADRENALINE LEVELS IN THE LUNG OF THE MALE RAT

Series	Monoamine oxidase	P	Serotonin	P	Nor adrenaline	P
Normal	54.75 ± 2.76		1.95 ± 0.24		0.11 ± .02	
3 Hour	19.68 ± 2.10	< .001	2.15 ± 0.19		0.16 ± .03	
20 Hour	18.62 ± 2.48	< .001	2.05 ± 0.18		0.13 ± .02	
36 Hour	18.61 ± 1.38	< .001	1.82 ± 0.12		0.08 ± .02	
72 hour	20.78 ± 1.47	< .001	2.26 ± 0.16		0.05 ± .01	< .001*

*Significantly lower.

(Significance was determined between normal and treated animals.)

Results are expressed as:

monoamine oxidase activity in $\frac{\mu l \text{ oxygen consumed/gm tissue/hour}}{10}$

serotonin and nor adrenaline in μg/gm.

The effect of Phenelzine on monamine oxidase, serotonin, and nor-adrenalin levels in the kidney of the male rat.

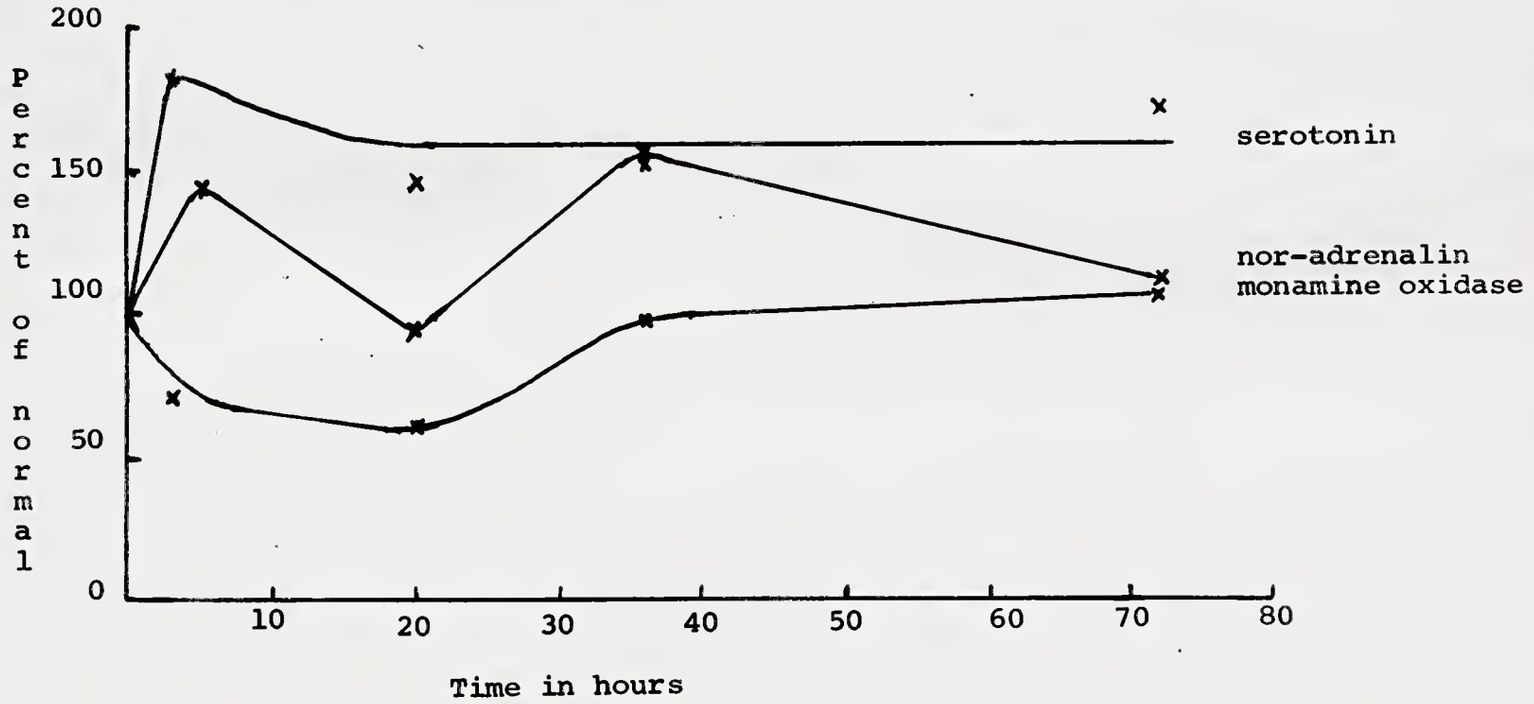

Figure 10

TABLE IV

THE EFFECT OF PHENELZINE ON MONOAMINE OXIDASE, SEROTONIN AND NOR ADRENALINE LEVELS IN THE KIDNEY OF THE MALE RAT

Series	Monoamine oxidase	P	Serotonin	P	Nor adrenaline	P
Normal	42.90 ± 2.71		0.54 ± .03		0.30 ± .05	
3 Hour	30.23 ± 2.83	< .001	0.99 ± .11	< .001	0.43 ± .03	< .01
20 Hour	26.64 ± 2.64	< .001	0.78 ± .06	< .001	0.28 ± .03	
36 Hour	41.34 ± 2.69		0.81 ± .03	< .001	0.48 ± .03	< .001
72 Hour	46.37 ± 1.58		0.92 ± .07	< .001	0.33 ± .03	

(Significance was determined between normal and treated animals.)

Results are expressed as:

monoamine oxidase activity in $\frac{\mu l \text{ oxygen consumed/gm tissue/hour}}{10}$

serotonin and nor adrenaline in µg/gm.

V. DISCUSSION

A brief discussion of the validity of the methods as well as the results obtained appeared necessary.

1. Discussion of the Methods

A. Monoamine Oxidase Estimation

The manometric method of Ehringer, Hornykiewicy and Lechner (144) was used with some modification for the determination of monoamine oxidase activity. The original method did not show volumes used and these were determined empirically. The principal modification was the use of the Huston-Martin technique (152) for the measurement of enzyme activity.

No effort was made to separate the monoamine oxidase enzyme from the tissues before the measurement of activity. Creasy (124) has investigated all known enzyme systems which might interfere with the manometric estimation of MAO and has concluded that such interference is minimal if indeed it occurs at all. In addition, the careful consideration of such factors as pH and substrate specificity undertaken in establishing the procedure will further reduce any interference by other systems.

It is therefore considered that the method for the estimation of MAO activity used in this work achieves a high degree of precision. Statistical analysis of the results obtained lends support to this conclusion because of the reproducibility and the low standard errors observed.

It is interesting to note that McGrath and Horita (148) using a colorimetric method reported similar results for brain and liver MAO activity by measuring the disappearance of substrate.

B. Serotonin and Nor Adrenaline Estimation

The method used for the extraction of serotonin and nor adrenaline is essentially the same as that developed by Shore and Olin (145) for the extraction of catechol amines. The volumes of butanol and heptane, as well as the shaking time, are modified from the original procedure. A series of experiments was performed to determine the volumes of these solvents necessary to extract both serotonin and nor adrenaline. Eight ml. of butanol was found to be sufficient to extract the amines and a ratio of 7 ml. heptane to 4 mls. butanol was found to be optimum in order to return the amines to the aqueous phase. A similar set of experiments was performed to find the optimum shaking time in order to extract the amines. Little change was noted between 15-minute and 30-minute shaking times when extracting the amines from the acid homogenate. Shaking longer than 30 minutes decreases the efficiency of the extraction due to the partial return of the amines to the aqueous phase (145). Similarly, 5 minutes was found to be optimum for shaking the acid and butanol-heptane mixtures.

The development of fluorescence of nor adrenaline by conversion to the trihydroxyindole compound was carried out by the same method used by Shore and Olin.

The addition of 0.3 mls. 12N HCl to 1 ml. of the acid extract in order to determine serotonin was first used by Bogdanski (149). Serotonin can be read directly in the 1 ml. extract; however, other substances, notably indoles, will also fluoresce at this pH. By lowering the pH, all fluorescence is due to 5-hydroxy indoles present. For practical purposes, all 5-hydroxy indoles found in tissue are reported as serotonin (149).

The use of fluorescence has greatly increased specificity and precision in estimating tissue amines. The reproducibility and agreement among the results would suggest that a satisfactory degree of precision was obtained by these methods.

2. Discussion of Results

A. Brain

The results obtained in the study on brain with regard to MAO inhibition produced by phenelzine agree closely with the published results of other workers. McGrath and Horita (148) reported a 94 per cent inhibition in two hours, compared with a 97.5 per cent inhibition in three hours (as shown in Table I). Chessin et al. (150) reported a significant depression of MAO activity in the brain of the mouse 24 days after the administration of phenelzine. McGrath and Horita (148) have shown that phenelzine is able to inhibit selectively brain MAO when compared with liver MAO. These results have been confirmed by this study.

The serotonin levels of the brain increased to approximately 162 per cent of normal in 3 hours. These results are similar to those obtained by Chessin et al. (150) in mice. These workers also found the greatest increases in brain 5-HT concentration occurred at the 3-hour time interval after injection. The gradual decrease of 5-HT to about 126 per cent of normal in 72 hours, as shown in Table I, was also observed by Chessin et al.

Although many MAO inhibitors have been studied in relation to changes in NA levels, phenelzine has apparently not been examined in this particular aspect of neurobiochemical research. Results obtained by this study indicate that NA levels do not change in the brain after a single dose of phenelzine. Funderburk et al. (88) have previously shown this to be true for other MAO inhibitors such as nialamide and tranylcypromine, although reports by others indicate that some MAO inhibiting agents, notably iproniazid, do raise central levels of NA (85-87,93,97). It appears that all MAO inhibitors do not increase nor adrenaline in the brain but do cause an increase in the concentration of serotonin. This latter effect seems to be the one common property shared by MAO inhibiting psychic energizers. Brodie (85) states that increases in activity are not correlated with the relatively rapid increases in concentration of serotonin. However, it has been shown that more time is required to elevate free serotonin levels than total serotonin levels (101,102).

It has also been shown that free NA levels do not increase following administration of iproniazid although the total NA concentration does rise (101). On the basis of the results obtained in this study and the evidence cited above, it would appear that MAO inhibiting agents produce mood elevation by increasing the concentration of brain serotonin.

B. Liver

Inhibition of liver MAO activity was produced in 3 hours following administration of phenelzine. This observation again confirms the work of McGrath and Horita (148). The increase in MAO activity to a supranormal level (133 per cent) in 72 hours could be explained by the increase in the amount of the substrate for the enzyme, that is, the increase in 5-HT. The liver contains the greatest amount of MAO of any organ in the body and the role of the liver in the detoxification of many compounds is well known. By the end of 72 hours after the administration of the monoamineoxidase inhibitor, the amine content of the various tissues studied, other than liver tissue, has shown a decrease to normal. It is possible that the increased concentration of the amine in these tissues which presumably was brought about by the inhibition of MAO, must be metabolized by the liver. The concentration of 5-HT would thus be far greater than normal and would serve as a stimulant to the enzyme system resulting in greater than normal activity.

Serotonin increases, following iproniazid, have been reported previously in the liver of the rat 4 hours after injection (66). In this study, serotonin levels remained elevated up to 20 hours, returned to normal in 36 hours and then rose significantly by 72 hours. The same explanation as was given for the increase in MAO activity at this time interval would seem to apply to the increase in 5-HT.

The concentration of nor adrenaline also rose in 3 hours in the liver indicating that MAO must play some role in the metabolism of this amine. It has previously been suggested that MAO is necessary for the metabolism of amines within tissues whereas catechol-O-methyl transferase is the main enzyme in the degradation of free or circulating amines (27). The findings in this report would tend to confirm this statement. In 20 hours, the NA content had returned to normal but rose again in 36 hours. An examination of these results shows that the figures are nearly identical, the 36-hour figure being just sufficiently greater to make it statistically significant. The slight difference between the figure obtained for the concentration of nor adrenaline at 20 hours and that obtained at 36 hours can probably not be considered important. Examination of the results throughout the time series would suggest that the increase in NA in 3 hours is followed by a gradual decrease to the normal level.

C. Lung

MAO activity dropped to 36 per cent of normal by 3 hours

and remained at this low level throughout the other time periods studied. Apparently MAO does not have as much biological significance in the lung as it does in some other tissues, notably the liver. In any case, the recuperative powers of MAO activity in lung tissue do not appear to be great.

Serotonin levels did not change in the lung following administration of the drug. Recent studies, using C^{14} labelled 5-hydroxytryptophan in the rabbit have shown that serotonin turnover is extremely slow in the lung and spleen (151). Since 5-HT levels did not rise after the enzyme which metabolizes the amine was inhibited, it would appear that either 5-HT is not synthesized in the lung or synthesis is very slow. It may be suggested that the lung is only a storage site for 5-HT.

Nor adrenaline levels gradually decreased to 45 per cent of normal in 72 hours in the lung. The NA content of the lung is largely, or solely, dependent on sympathetic innervation and the lung is poorly innervated by the sympathetic nervous system (69). Several MAO inhibitors have been shown to function as ganglionic blocking agents (140,141) and to produce postural hypotension (137). Such ganglionic blocking would therefore decrease the NA present and the gradual decrease in concentration of nor adrenaline observed in the lung may well be attributed to this effect.

D. Kidney

Kidney MAO activity was found to be significantly depressed 3 hours after injection of the drug. MAO activity was further inhibited at the end of 20 hours. Normal values were observed in the other two time intervals investigated. Kidney MAO activity thus exhibited a return to normal much more rapidly than lung or brain but not as rapid as was observed in the liver.

The concentration of serotonin in the kidney increased rapidly to a value approximately 183 per cent of normal at the end of 3 hours and maintained this higher than normal value throughout the investigation (see Table IV). There was no suggestion of a decrease towards normal in any of the time intervals studied. It should be noted that this was the greatest and most prolonged increase in 5-HT concentration observed in any tissue. The physiological implications of 5-HT in the kidney have been given some consideration (46,47) but no satisfactory hypothesis has been advanced. The findings reported here are of interest but no conclusions can be drawn.

Nor adrenaline showed considerable variation at the different time intervals studied. Normal values were observed at 20 and 72 hours but higher than normal levels were found after 3 and 36 hours. The increase in NA content after 3 hours was similar to that seen in the liver and could be explained in the same manner. The rapid decline in the NA concentration to a normal value followed by an equally rapid elevation after 36

hours is not, however, as easily explained. Nor adrenaline, and its metabolites, are excreted by the kidney and therefore not all of the NA extracted will necessarily be endogenous to the organ. That nor adrenaline released in the body and carried to the kidney is first stored and then excreted may be a possibility. Such a hypothesis, however, would require more evidence than is presented in this study.

VI. SUMMARY AND CONCLUSIONS

1. A method for the simultaneous extraction of serotonin and nor adrenaline was presented which is simpler and as efficient as the original method proposed by Shore and Olin (145).
2. The Huston-Martin apparatus (152) was applied to the manometric measurement of monoamine oxidase activity.
3. The administration of phenelzine by intraperitoneal injection was found to inhibit the monoamine oxidase activity of the brain, liver, lung and kidney in the male rat.
4. The concentration of serotonin was found to be increased significantly in all tissues studied, with the exception of the lung.
5. It was postulated that the lung serves as a storage site for serotonin.
6. The concentration of nor adrenaline did not change in the brain at any of the time intervals studied. It was suggested that the pharmacological effects of the monoamine oxidase inhibitor are mediated through serotonin and not through nor adrenaline.
7. The concentration of nor adrenaline was found to decrease in the lung. The action of the inhibitor as a ganglionic blocking agent was suggested as the reason for this depletion.

8. The increased concentration of nor adrenaline which was noted in the liver and kidney at both the 3 hour and 36 hour time intervals suggested that monoamine oxidase was necessary for the metabolism of the amine in tissue.
9. It was suggested that the supranormal activity of monoamine oxidase noted in liver at the 72 hour time interval was due to an increased concentration of the substrate serotonin which resulted in a stimulation of the enzyme system.

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APPENDIX

Table i

MONOAMINE OXIDASE LEVELS IN THE BRAIN OF NORMAL AND TREATED RATS

Figures represent $\frac{\mu l\ O_2 \text{ consumed/gm. wet weight of tissue/hr.}}{10}$.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

Normal		Hours 3	20	36	72
34.35		00.00	39.80	39.61	37.68
25.73		00.00	40.81	32.95	40.24
36.99		00.00	47.20	23.78	23.92
60.83		00.00	40.54	30.80	25.84
48.15		00.00	37.44	39.74	32.34
33.41		00.00	26.75	22.00	32.66
45.07		00.00	41.20	29.08	35.27
51.65		00.00	18.28	31.16	38.34
68.09		00.00	31.74	25.02	31.00
34.50		00.00	18.85	30.02	32.78
52.46		6.64	34.37	22.12	34.95
34.18		6.64	34.35	26.98	30.27
43.78	(Mean)	1.10	34.28	29.44	33.19
3.68	(S.E.M.)	0.75	2.60	1.72	0.67

Table ii

MONOAMINE OXIDASE LEVELS IN THE LIVER OF NORMAL AND TREATED RATS

Figures represent $\frac{\mu l\ O_2 \text{ consumed/gm. wet weight of tissue/hr.}}{10}$

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

		Hours			
Normal		3	20	36	72
68.05		48.21	98.99	26.46	88.19
52.30		56.84	43.98	28.38	88.42
71.51		48.01	58.22	51.49	94.06
67.07		35.82	70.85	63.44	101.23
89.66		48.25	66.41	84.75	94.52
81.60		59.76	66.10	57.67	105.75
52.95		43.73	62.14	72.11	109.77
83.80		37.48	42.56	84.69	75.53
71.16		44.00	44.63	69.87	93.31
63.12		35.16	50.16	71.27	78.65
67.07		38.87	55.98	70.72	85.61
56.50		48.09	68.05	77.57	86.07
68.73	(Mean)	46.39	60.67	63.20	91.43
3.43	(S.E.M.)	1.97	4.56	5.59	3.01

Table iii

MONOAMINE OXIDASE LEVELS IN THE LUNG OF

NORMAL AND TREATED RATS

Figures represent $\frac{\mu l\ O_2 \text{ consumed/gm. wet weight tissue/hr.}}{10}$

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

Normal		Hours 3	20	36	72
38.95		17.68	21.38	21.86	18.43
67.73		14.87	41.56	13.76	15.98
58.91		16.67	20.00	22.06	13.16
60.47		13.15	6.01	28.49	23.52
61.97		22.87	10.00	18.41	31.34
47.93		16.76	12.23	17.70	18.32
57.74		17.02	6.43	15.89	21.36
41.08		22.08	21.52	19.61	18.51
43.90		39.77	22.65	10.34	24.87
65.45		24.60	13.14	17.58	25.57
58.20		13.15	28.65	15.67	15.80
54.78		17.54	19.86	22.44	22.59
54.75	(Mean)	19.68	18.62	18.61	20.78
2.76	(S.E.M.)	2.10	2.48	1.38	1.47

Table iv

MONOAMINE OXIDASE LEVELS IN THE KIDNEY

OF NORMAL AND TREATED RATS

Figures represent $\frac{\mu l\ O_2 \text{ consumed/gm. wet weight tissue/hr.}}{10}$.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

Normal		Hours 3	20	36	72
32.81		30.22	18.80	59.25	54.94
35.33		30.58	40.39	49.05	41.12
42.28		10.48	11.87	30.79	39.21
44.05		23.56	39.14	43.44	42.93
52.33		34.62	23.58	37.44	40.01
38.84		34.64	27.01	48.27	61.98
58.96		21.43	33.04	40.33	47.19
55.71		47.03	14.75	25.75	36.15
44.91		37.77	19.78	30.66	33.88
44.51		41.37	29.85	47.28	50.10
38.43		27.85	28.77	42.90	53.45
26.63		23.22	32.81	41.00	56.58
42.90	(Mean)	30.23	26.64	41.34	46.37
2.71	(S.E.M.)	2.83	2.64	2.69	1.58

TABLE v

SEROTONIN LEVELS IN THE BRAIN OF NORMAL AND TREATED RATS

Figures represent μg. serotonin/gm. wet weight tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hours			
Normal	3	20	36	72
0.70	0.84	1.36	0.84	0.99
0.55	0.84	1.46	0.96	0.93
0.47	1.25	0.96	0.77	0.85
0.54	1.46	0.75	1.19	0.81
0.67	0.87	1.00	1.09	0.86
0.66	1.42	1.35	1.16	0.81
0.58	1.08	0.89	0.90	0.80
0.61	1.14	0.94	1.14	0.78
0.73	1.13	0.80	0.86	0.79
0.70	1.13	1.00	0.95	0.81
0.74	1.10	0.89	0.90	0.84
0.92	0.98	0.80	0.87	0.99
0.80				
0.64				
0.83				
0.68				
0.68 (Mean)	1.10	1.02	0.97	0.86
0.03 (S.E.M.)	0.06	0.07	0.04	0.02

Table vi

SEROTONIN LEVELS IN THE LIVER OF

TREATED AND NORMAL RATS

Figures represent μg. serotonin/gm. wet weight tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hours			
Normal	3	20	36	72
0.73	0.77	0.54	0.69	0.47
0.61	0.71	0.99	0.55	0.54
0.61	0.80	0.93	0.43	0.54
0.58	1.35	0.93	0.39	0.67
0.85	0.70	0.90	0.51	0.82
0.83	0.73	1.00	0.84	0.52
0.51	1.63	0.78	0.76	0.63
0.64	1.21	1.24	0.76	0.95
0.59	0.58	1.02	0.51	1.53
0.51	0.63	1.11	0.64	1.20
0.51	0.58	0.64	0.58	1.20
0.48	0.56	0.91	0.58	1.20
0.66				
0.63				
0.57				
0.52				
0.61 (Mean)	0.85	0.92	0.60	0.86
0.03 (S.E.M.)	0.10	0.06	0.04	0.10

TABLE vii

SEROTONIN LEVELS IN THE LUNG OF

NORMAL AND TREATED RATS

Figures represent μg. serotonin/gm. wet weight tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hours			
Normal	3	20	36	72
1.85	1.40	1.79	1.15	1.30
1.58	3.15	1.98	2.33	2.00
1.90	2.15	2.04	2.49	2.10
1.84	2.63	3.38	1.55	2.93
1.33	2.86	1.25	1.11	2.07
1.98	2.44	1.62	1.52	2.39
2.14	1.65	2.03	1.90	2.36
2.30	2.04	2.10	1.80	1.78
1.84	2.79	1.39	2.10	2.10
2.70	2.24	2.88	2.16	2.79
2.18	1.28	1.68	1.98	1.92
2.11	1.18	2.42	1.75	3.40
1.86				
1.83				
1.84				
1.90				
1.95 (Mean)	2.15	2.05	1.82	2.26
0.24 (S.E.M.)	0.19	0.18	0.12	0.16

TABLE viii

SEROTONIN LEVELS IN THE KIDNEY OF

NORMAL AND TREATED RATS

Figures represent µg. serotonin/gm. wet weight tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hours			
Normal	3	20	36	72
0.69	0.60	0.74	0.76	0.67
0.74	0.65	0.58	0.97	1.37
0.59	1.79	1.18	0.74	0.69
0.75	0.84	0.67	0.94	1.07
0.47	1.08	1.13	0.65	1.25
0.57	0.95	0.70	0.79	0.82
0.46	0.64	0.78	0.67	1.02
0.50	0.98	0.51	0.76	0.90
0.54	0.86	0.61	0.99	0.85
0.46	0.74	0.71	0.76	0.95
0.56	1.48	1.00	0.82	0.63
0.41	1.29	0.76	0.85	0.81
0.49				
0.46				
0.47				
0.49				
0.54 (Mean)	0.99	0.78	0.81	0.92
0.03 (S.E.M.)	0.11	0.06	0.03	0.07

TABLE ix

NOR ADRENALINE LEVELS IN THE BRAIN OF

NORMAL AND TREATED RATS

Figures represent µg. nor adrenaline/gm. wet weight of tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

Normal	Hour 3	20	36	72
0.41	0.37	0.21	0.21	0.33
0.28	0.31	0.24	0.33	0.34
0.21	0.41	0.32	0.33	0.38
0.23	0.56	0.26	0.29	0.39
0.16	0.35	0.36	0.47	0.38
0.17	0.32	0.32	0.45	0.24
0.12	0.39	0.28	0.44	0.17
0.33	0.40	0.30	0.44	0.26
0.43	0.32	0.25	0.27	0.45
0.46	0.30	0.33	0.30	0.20
0.34	0.33	0.34	0.32	0.30
0.45	0.36	0.27	0.26	0.25
0.36				
0.31				
0.41				
0.42				
0.32 (Mean)	0.37	0.29	0.34	0.31
0.03 (S.E.M.)	0.02	0.01	0.01	0.03

TABLE x

NOR ADRENALINE LEVELS IN THE LIVER

OF NORMAL AND TREATED RATS

Figures represent µg. nor adrenaline/gm. wet weight of tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

Normal	Hour 3	20	36	72
0.07	0.21	0.17	0.12	0.01
0.09	0.22	0.33	0.14	0.14
0.19	0.25	0.16	0.20	0.10
0.16	0.27	0.13	0.18	0.18
0.05	0.14	0.15	0.29	0.15
0.24	0.17	0.10	0.28	0.06
0.14	0.46	0.24	0.29	0.08
0.02	0.25	0.30	0.35	0.21
0.16	0.27	0.15	0.15	0.25
0.13	0.28	0.20	0.18	0.17
0.16	0.33	0.14	0.15	0.34
0.19	0.27	0.16	0.23	0.24
0.19				
0.23				
0.15				
0.13				
0.14 (Mean)	0.26	0.19	0.21	0.16
0.02 (S.E.M.)	0.02	0.02	0.02	0.03

TABLE xi

NOR ADRENALINE LEVELS IN THE LUNG

OF NORMAL AND TREATED RATS

Figures represent µg. nor adrenaline/gm. wet weight of tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hour			
Normal	3	20	36	72
0.10	0.22	0.11	0.06	0.02
0.18	0.27	0.16	0.03	0.02
0.14	0.20	0.19	0.09	0.02
0.17	0.22	0.26	0.00	0.06
0.11	0.15	0.07	0.10	0.10
0.27	0.23	0.04	0.12	0.08
0.16	0.06	0.25	0.16	0.10
0.12	0.24	0.10	0.11	0.10
0.00	0.03	0.05	0.00	0.03
0.00	0.00	0.16	0.09	0.00
0.05	0.18	0.12	0.05	0.04
0.10	0.10	0.06	0.16	0.05
0.09				
0.04				
0.07				
0.18				
0.11 (Mean)	0.16	0.13	0.08	0.05
0.02 (S.E.M.)	0.03	0.02	0.02	0.01

TABLE xii

NOR ADRENALINE LEVELS IN THE KIDNEY

OF NORMAL AND TREATED RATS

Figures represent µg. nor adrenaline/gm. wet weight of tissue.

Treated animals were injected with β-phenylethylhydrazine 3, 20, 36 and 72 hours before sacrificing.

	Hour			
Normal	3	20	36	72
0.32	0.31	0.40	0.35	0.19
0.36	0.35	0.43	0.41	0.30
0.34	0.37	0.30	0.54	0.28
0.35	0.34	0.20	0.66	0.31
0.24	0.35	0.29	0.44	0.21
0.22	0.53	0.14	0.63	0.26
0.26	0.39	0.29	0.45	0.39
0.29	0.36	0.29	0.48	0.28
0.14	0.37	0.17	0.38	0.39
0.20	0.46	0.34	0.36	0.46
0.28	0.65	0.25	0.47	0.37
0.33	0.63	0.27	0.54	0.49
0.31				
0.35				
0.39				
0.37				
0.30 (Mean)	0.43	0.28	0.48	0.33
0.05 (S.E.M.)	0.03	0.03	0.03	0.03

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b4 this thesis

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